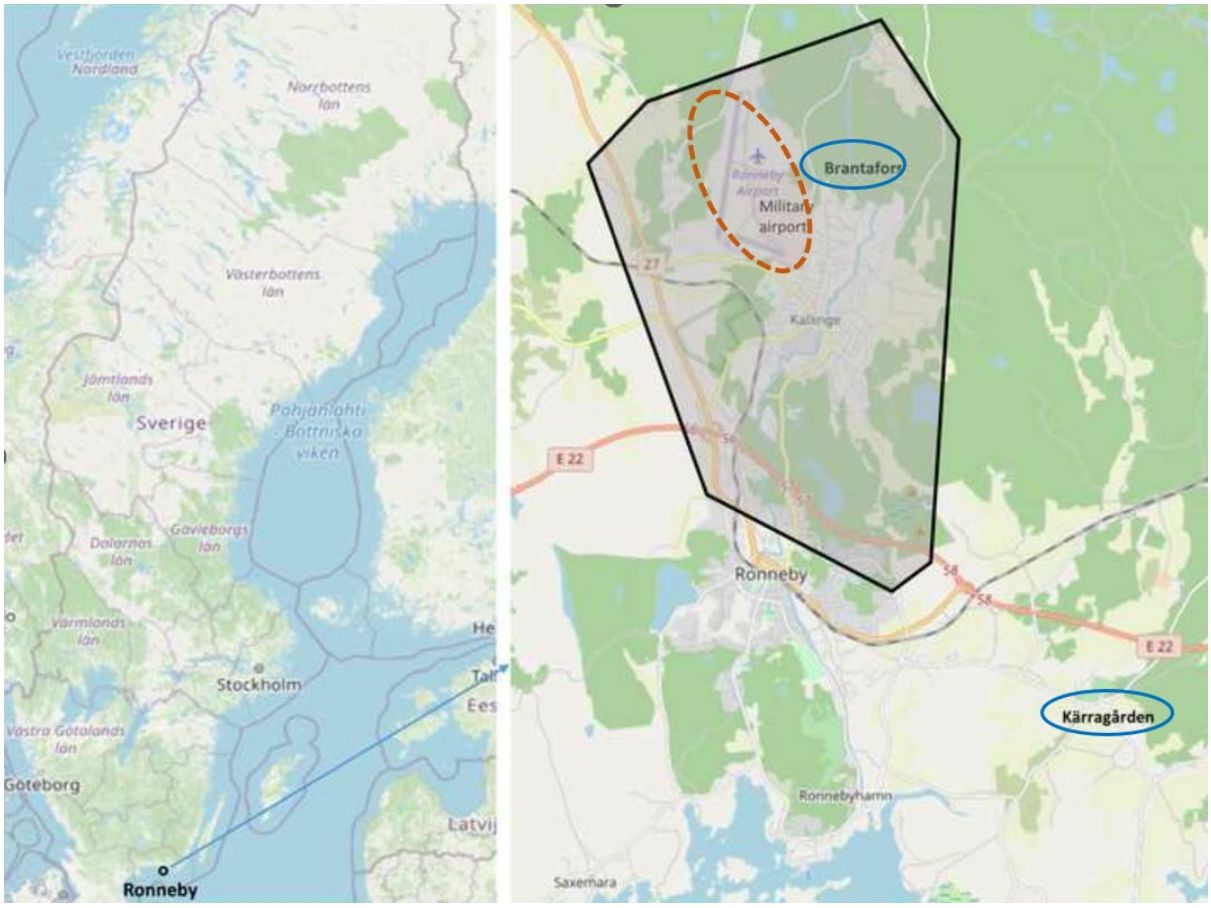
Report 2024-03-05

Ronneby PFAS Research Programme

A summary of research 2014 - 2023



Editors

Professor Kristina Jakobsson, Occupational and Environmental Medicine, Department of Medicine, University of Gothenburg, Gothenburg, Sweden

Associate Professor Christel Nielsen, Occupational and Environmental Medicine, Department of Laboratory Medicine Lund, Lund University, Lund, Sweden.

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Ronneby PFAS Research Programme (RPRP)

Eva Andersson, Axel Andersson, Magdalena Levandowski, Christian Lindh, YiYi Xu, Annelise Blomberg.
Carina A Nilsson, Eva M Andersson, Christel Nielsen, Kristina Jakobsson, Tony Fletcher, Matilda Ebel, Erika Norén.
Carmela Miniscalco, Charlotte Stübner, Huiqi Li, Sofia Hammarstrand, Ying Li, Daniela Pineda

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kristina.jakobsson@amm.gu.se christel.nielsen@med.lu.se tony.fletcher@lshtm.ac.uk

christian.lindh@med.lu.se

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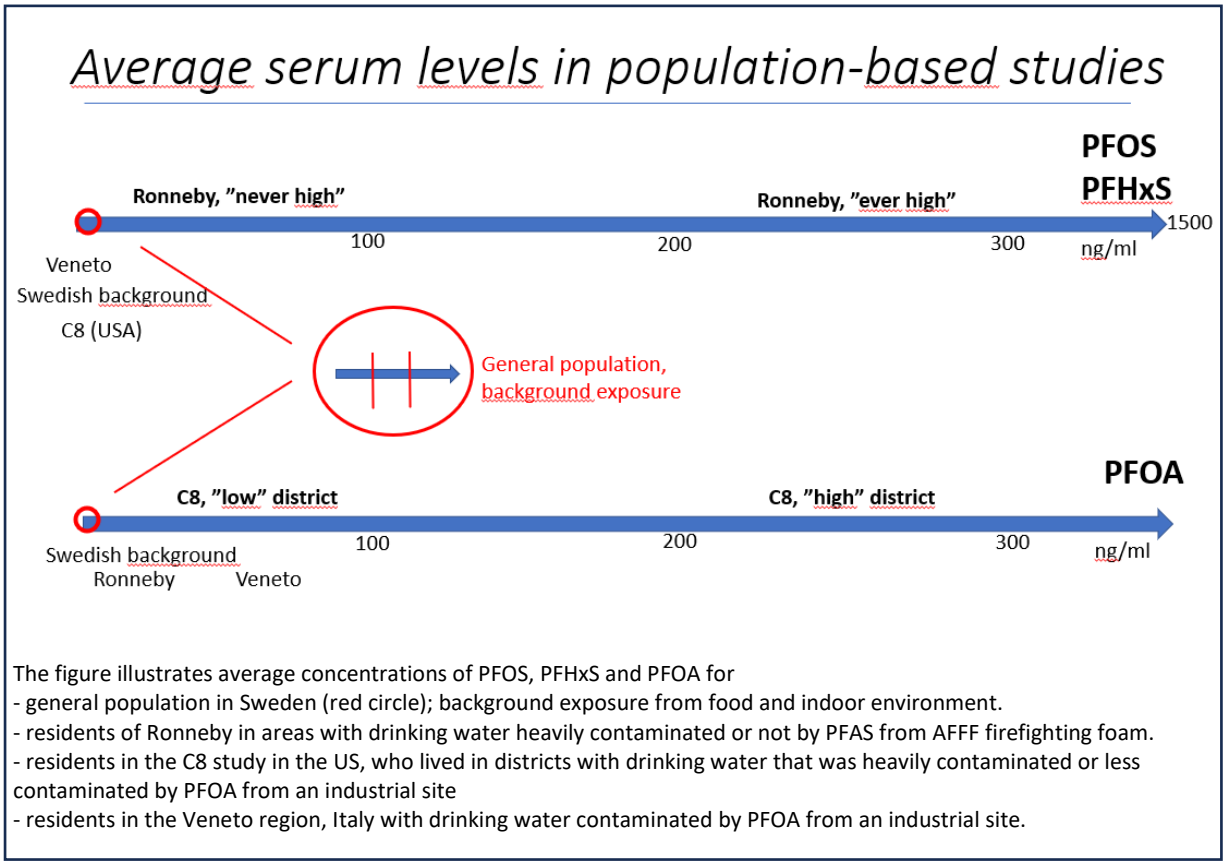
Summary

It has been ten years since it was discovered that the drinking water in one of the waterworks in Ronneby, which supplied approximately 1/3 of the households with drinking water, was very heavily contaminated with PFAS. Although the contaminated supply was immediately shut off and replaced with clean water, everyone who had been drinking the contaminated water for a long time still has elevated levels of PFAS in their bodies and will continue to do so for a long time to come. Women with elevated levels of PFAS in their bodies will pass some PFAS to their children during pregnancy and breastfeeding.

The situation in Ronneby is unique in several ways. First, the PFAS levels in the water were very high, especially for the substances PFOS and PFHxS, and second, there were clear differences within the municipality as only one of the waterworks had heavily contaminated water. This, together with the good opportunities for research in Sweden, has made it possible to study the health effects of PFASs over a very wide exposure range. For the substance PFOA, there are studies from the USA with very high exposure from drinking water (the so-called C8 studies). However, the majority of all epidemiological studies in general populations have been conducted at much lower exposures and studies at high exposures to PFOS and PFHxS outside Ronneby are completely lacking.



The figure illustrates average concentrations of PFOS, PFHxS and PFOA for
- general population in Sweden (red circle); background exposure from food and indoor environment.
- residents of Ronneby in areas with drinking water heavily contaminated or not by PFAS from AFFF firefighting foam.
- residents in the C8 study in the US, who lived in districts with drinking water that was heavily contaminated or less contaminated by PFOA from an industrial site
- residents in the Veneto region, Italy with drinking water contaminated by PFOA from an industrial site.

A broad research programme to investigate the health effects of PFAS exposure started within weeks of the contamination becoming known. In this report, we summarise the results of all the sub-studies carried out so far and describe the infrastructure for further research that has been built up.

In some cases, our results have confirmed previous findings, observed at background levels and/or in the C8 studies with high PFOA exposure. These include elevated cholesterol levels and increased risk of kidney cancer. Such consistency reinforces that there is a real causal relationship with PFAS exposure. However, it does not seem to be the case that a much higher exposure to PFAS has resulted in a much higher risk.

In other cases, neither our studies nor the C8 studies can confirm findings from different background exposure groups. This applies, for example, to impacts on thyroid hormones and childbirth weight. Another example where consistency is lacking is that previous research has shown that children's antibody responses after vaccinations are reduced by PFAS, while we could not see any such effect among adults with very high PFAS exposure.

But we have also found elevated risks for several conditions for which there were previously only limited and conflicting results - such as increased risk of osteoporosis fractures, diabetes, the women's disease PCOS, and an increased incidence of language disorders in children. These are observations that need to be confirmed in more studies.

High PFAS exposure and associations with health effects in Ronneby, Sweden

Common conditions:
We have found an increased incidence of type 2 diabetes.
We have found an increased risk of fractures associated with osteoporosis.
We have found an increased risk of elevated blood cholesterol levels.
We have found an increased risk of language impairment among girls, but not among boys.
We have found an increased risk of a shortened breastfeeding period.

We have *not* found an increased risk of common cancers such as breast cancer, prostate cancer, and bowel cancer.
We have *not* found an increased risk for low birth weight babies (<2500g) and only a minor effect on birth weight (increased among girls, decreased among boys).

Less common conditions
We have found an increased risk of kidney cancer.
We have found an increased risk of the female disease PCOS, but *not* an increased risk of endometriosis.

We have *not* found an increased risk of pregnancy complications.
We have *not* found an increased risk of thyroid disease.
We have *not* found an impaired antibody response after vaccination in adults.
We have *not* found an increased risk of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease.

Unusual conditions:
We have found an increased risk of testicular cancer.

Is more research needed in Ronneby?

We see two main areas where we need to do more. One is to follow the next generation because PFASs are transferred from mother to child during pregnancy and breastfeeding. We need to follow children's development throughout their childhood in terms of both physical development and brain development. The Ronneby Mother-Child Cohort is the basis for such studies.

The second important area is to expand the register studies to include the entire population of Blekinge, not just those who have lived in Ronneby. Then, we additionally can get more knowledge about the health effects among those with moderately elevated PFAS exposure, i.e. those living in Ronneby, but never with contaminated drinking water at home. They are more PFAS exposed than those who lived in the other municipalities in the county with only background exposure.

Our research results have implications for international and national regulation of PFAS use. Epidemiological studies are the basis for the new guidelines and limit values for drinking water, proposed by authorities in Europe and North America. These values are intended to protect an entire population from any harmful effects related to lifetime exposure.

However, data from moderately and highly exposed groups are also needed as more and more areas with moderately elevated PFAS exposure due to contaminated drinking water and food are being identified. For risk assessment and risk communication, better knowledge of the relationship between the magnitude of exposure (dose) and various health effects is needed. Our research is at the forefront of studies of health effects in populations exposed to high levels of PFAS.

Introduction

In late autumn 2013, high levels of PFAS were detected in soil and groundwater in Ronneby. It soon became clear that the drinking water was very heavily contaminated in one of the municipality's two waterworks. The population was informed, and the contaminated water was disconnected on December 16, 2013. The following week, the environmental administration in Ronneby contacted the Occupational and Environmental Medicine Clinic in Lund (AMM). It was agreed to investigate human exposure level in the municipality rapidly and a pilot study examining children from a school in the centre of the area with contaminated drinking water and a school in a completely different part of the municipality was planned.

The study, which was completed in early March 2014, showed large differences between the children's PFAS levels in their blood based on which school they attended. This was the start of a major campaign, in which all Ronneby residents were offered to have their PFAS levels analysed free of charge. Starting in May 2014 and continuing in 2015 - 2016, AMM took blood samples at health centres, schools, workplaces, and the town hall in Ronneby. This work was financed in its entirety within the clinic's regular activities in the southern healthcare region, which also includes Blekinge.

At the same time, research was prepared on how PFAS exposure could have affected the health of the exposed population. With a large emergency grant from the government research council FORMAS, a large number of research studies were initiated. In the years that have passed, the researchers at the universities of Lund and later also Gothenburg and the London School of Hygiene and Tropical Medicine in the joint Ronneby PFAS Research Program (RPRP) have received several large project grants from the Swedish government research councils (see also p. 37). In this report, we describe the resources for ongoing and future research that have been built up within the RPRP and the results of the scientific studies that have been reported so far. Ongoing studies are also briefly described.

When the PFAS contamination in Ronneby was discovered, there was very little data from human epidemiological studies on different PFAS, and almost none on the PFAS exposure mixture in Ronneby. The situation today is very different. Many studies have been done, especially in populations with general background exposure from food, drinking water, consumer goods and indoor environments. Together with studies among PFAS-exposed workers in the USA, the population around a polluting industry (often called the C8 studies[1]) and the population in Veneto, Italy, the studies in Ronneby are the only studies to date with a very wide exposure range, from background levels to very high levels. However, the pattern of PFAS exposure is different in these populations. PFOA[2] from industrial contamination dominated in the C8 and Italian studies while high levels of PFOS[3] and PFHxS[4] from firefighting foam contaminating drinking water dominated in Ronneby. The significance of these differences on the health study results, is still unclear.

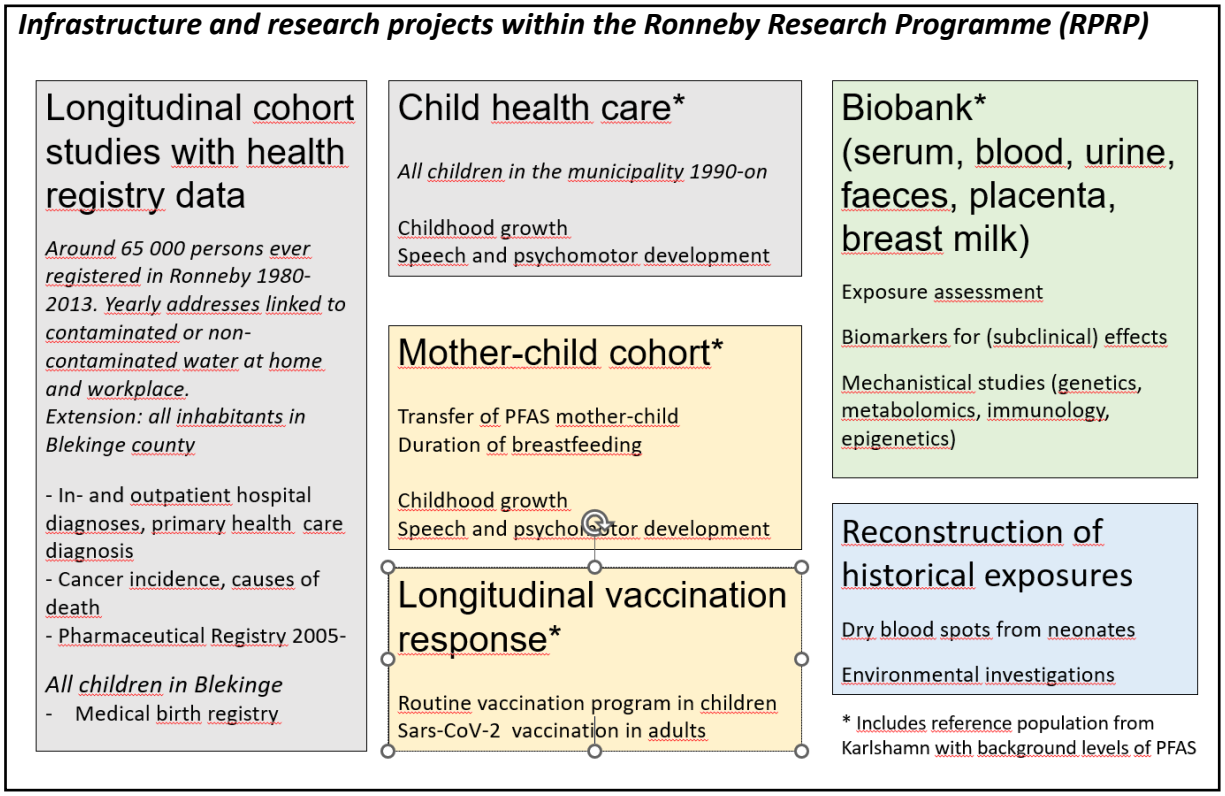
Research infrastructure in Ronneby PFAS Research Programme (RPRP)

There are unique opportunities for research in Sweden because data from different registers and databases can be linked through personal identification numbers. These links are carried out by the Statistics Sweden (the Swedish statistics authority, or SCB). Researchers never receive any identity

[1] http://www.c8sciencepanel.org/
[2] perfluorooctanoate
[3] perfluorooctasulfonate
[4] perfluorohexasulfonate

information, only an individual unique study serial number. SCB stores the key between the serial number and the personal number. In this way, individuals' confidentiality is protected. All our studies are authorised by the Regional Ethical Review Board or the Ethical Review Authority, in some cases also by the Swedish Medical Products Agency.

The content of the research infrastructure built under the RPRP is briefly described below.



Modelled population exposure to PFAS

For classifying exposure in the epidemiological studies, the following sources of information have been used:

- Annual data from Ronneby Miljö och Teknik AB on the distribution of drinking water from the waterworks in Brantafors and Kärragården to all properties in Ronneby municipality.
- Annual data on civil registration address, which SCB has linked to water distribution data.
- Annual data on workplace address, linked by Statistics Sweden to water distribution data.

This address data is the basis for categorising the annual PFAS exposure of individuals.

- Background exposure: Never registered in Ronneby municipality.
- Middle group: Ever registered in Ronneby municipality, but never at an address with PFAS-contaminated drinking water.
- High exposure: Ever registered in Ronneby municipality at an address with PFAS-contaminated drinking water.

Furthermore, additional categories have been created, such as different time windows accounting for the increasing water contamination over time, and the number of years at the address with contaminated water.

Measured population exposure to PFAS

During open sampling in Ronneby in 2014-2016, PFAS levels were measured in the blood of about 3,500 residents of Ronneby. In 2016, a comparison group in the neighboring municipality Karlshamn,

where the drinking water had not been PFAS-contaminated, was also recruited and PFAS levels were measured in their blood.

Blood and urine samples were analysed at the laboratory at the department of Occupational and Environmental Medicine, Lund University/Region Skåne using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Detailed descriptions of the method, which covered 16 different PFAS substances, have been published. For quality control, control samples and blanks were included in the analysis. The laboratory participates in several quality control programmes such as the HBM4EU project (2018-2019) and twice a year in a quality control program (G-EQUAS) coordinated by the University of Erlangen-Nuremberg, Germany. Methods have also been developed to also analyse PFAS in placentas and from dried blood spots from neonates on paper (so-called PKU samples).

Analyses of PFAS in breast milk are carried out in collaboration with researchers from the Norwegian Institute of Public Health in Oslo, Norway. PFAS in stool samples are analysed by researchers at Örebro University, Sweden.

Databases for register studies

Study groups

Data on individuals included in the register studies come from Statistics Sweden. We receive pseudonymised[5] information on address by year (residence, work), gender, age, highest level of education and other socio-demographic variables.

Our *first cohort*[6] included 63,074 persons who were registered in the municipality of Ronneby anytime from 1980 to 2013.

Our *expanded cohort* included everyone who had been registered in Blekinge anytime from 1980 to 2013, as well as the children born to the women in the cohort. A total of 361,950 individuals were included.

A *birth cohort* included 50,697 children born between 1985 and 2013. The database contains data for children born in Blekinge, but also data for children born outside Blekinge if the mother previously lived at least one year in Ronneby. The birth cohort will be updated with more years (children born until 2023 and on).

An *early childhood cohort* included 15,383 children born in Blekinge during the period 1998-2013, who have been registered in the county for at least one year between the ages 0 and 7 years. Their language development was followed via screening at the child healthcare services and follow-up at the Blekinge Hospital's speech therapy clinic.

Register data on health outcomes

Information on health outcomes in a cohort can be obtained from a variety of national and local health records, subject to ethical approval and permission from the record holder. The registers we have used so far are:

- Diagnoses from national health care registers (inpatient hospital care since 1985, outpatient hospital care since 2002).
- Cause of death register (start 1953)
- Cancer Registry (start 1958)
- Diagnoses in primary care in Blekinge (start 2004)
- Purchase of prescription drugs (start mid-2005)

[5] The data is pseudonymised by replacing the personal numbers by coded serial numbers, to which only Statistics Sweden has the key.

[6] A cohort is a well-defined group of individuals who are followed up over time.

- Information from the Medical Birth Register (start 1978)
- Diagnostic register at Blekinge Hospital (start 1998)

Information from child health services in Blekinge

We have a database that includes medical records, particularly on breastfeeding and growth, from BVC for 2,449 Ronneby children and 123 randomly selected children from Karlshamn, born between 1999 and 2009.

Biobank

We have a large biobank for long-term storage of biological samples at -80◦ C. The biobank was established in connection with the open sampling in Ronneby in 2014. Only samples where the person has given consent for future research are in the biobank. Researchers outside the RPRP group can request access to de-identified sample results or sample material. Where applicable, ethical authorisation is required.

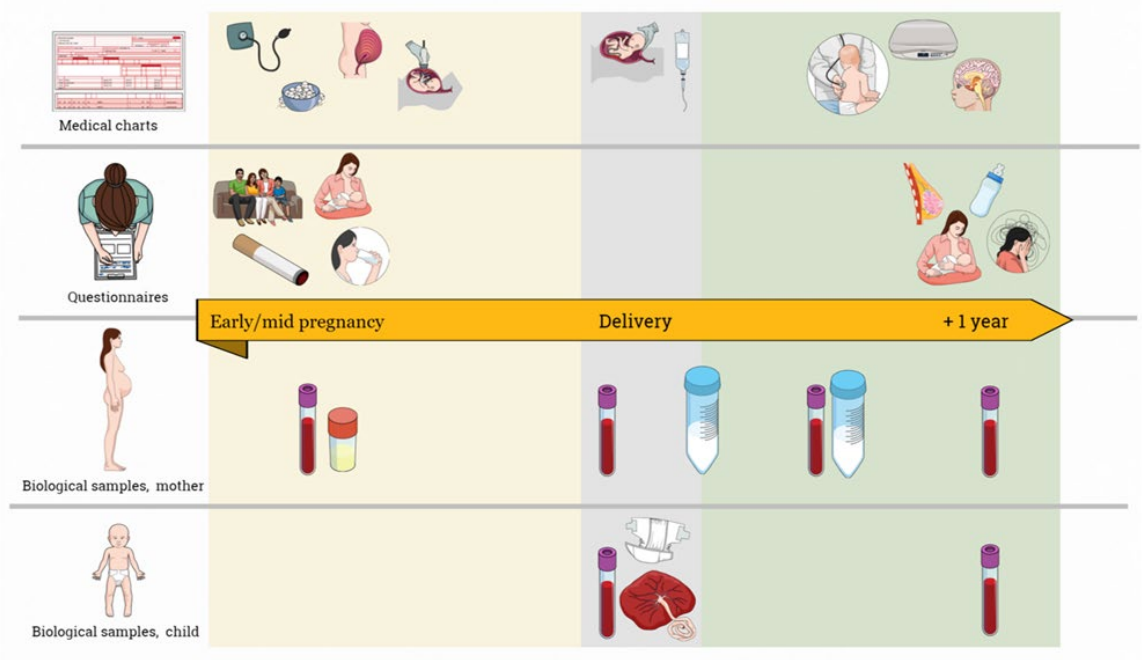
Blood tests (serum, whole blood)
3,507 persons who gave blood for PFAS testing on at least one occasion between 2014 and 2016, and who authorised the use of samples for future research.
182 former blood donors who participated in a follow-up survey in 2016.
114 persons who provided up to 10 repeat samples between 2014 and 2019.
309 persons from Ronneby who gave a second follow-up blood sample in 2021
226 persons from Karlshamn (comparison group) who provided blood samples in 2016.
47 persons from Karlshamn (comparison group) who provided a second blood sample in 2021.
357 adults from Ronneby and Karlshamn who participated in a vaccine study in 2022.

Other samples in the biobank
Urine and faeces samples from a small group of adults.
Blood samples, placentas, breast milk and baby feces from the Mother-Child cohort participants.
Small, punched samples from the PKU biobank at Karolinska Institutet[7] are used to analyse PFAS levels in the blood of 288 children born between 1985 and 2013. The blood is extracted and the extract can be stored for up to 2 years.

[7] https://www.karolinska.se/for-vardgivare/karolinska-universitetslaboratoriet/centrum-for-medfodda-metabola-sjukdomar/pku-biobank/

The Mother-Child Cohort and its biobank

Between 2015 and 2020, 263 pregnant women were recruited to the Ronneby Mother-Child cohort in collaboration with maternal health services in Ronneby and Karlshamn (the latter representing background exposure). The women and their children have so far been followed from mid-pregnancy until the child is one year old. The cohort will be used to study how PFAS exposure has affected the health of pregnant women and young children, as well as the growth and development of the children during preschool age and beyond (see further page 33).



The Ronneby Mother-Child cohort includes an extensive biobank, a database with repeated questionnaires completed by carers, and information from medical records from maternity and pediatric health services.

Biological samples and questionnaire data collected from the participants in the Ronneby Mother-Child cohort.

Pregnancy		**Delivery**		**Breast feeding**		**Child 1 year**	
Sample type	*Number*	*Sample type*	*Number*	*Sample type*	*Antal*	*Sample type*	*Number*
Blood, mother	248	Blood, mother	213	Breastmilk, mature	100	Blood, child	133
Urine, mother	105	Blood, umbilical cord	211				
		Placenta	178	Breastmilk, repeated sampling	11 mothers		
		Faeces, child	6				
		Colostrum[8]	95				
Questionnaire data							
Family situation						Breastfeeding, diet	
Smoking						Breastfeeding attitudes	
Medical drug use						PFAS-related stress	
Parity						Personality	
Breastfeeding experience							

[8] Colostrum is the first breastmilk that is produced in the days after delivery.

Epidemiological studies

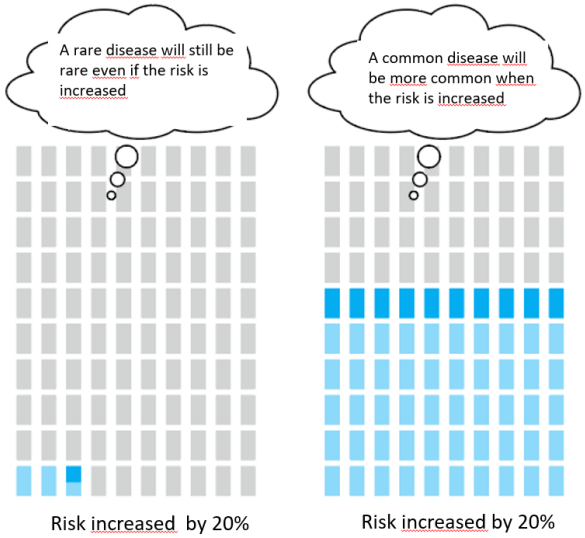
An epidemiological study examines the frequency of different conditions in groups. Often the aim is to find out if there is an association between exposure to a suspected risk factor and the outcome (i.e. the health condition), after correcting for other possible differences such as age.

In a *cross-sectional study,* exposure and outcome are measured simultaneously. It is possible to describe the relationship (association) between exposure and outcome, but there is some doubt about cause and effect as it is not clear if the exposure came first. Our biomarker studies, such as examining the association between the measured PFAS level in the blood and the simultaneously measured level of blood lipids, are one such example. However, by comparing how possible associations look in groups with different levels of exposure, we can still get an idea of whether there could be a causal relationship - does there seem to be more disease at higher levels of exposure?

In a *longitudinal* study, you first have a measure of exposure and then observe the outcome. This makes it possible to make more reliable statements about causal relationships. Our register studies are such examples. We have data on each person's annual home address since 1980, which is linked to data on the drinking water at the address (contaminated or not). Each person is followed over time in medical records. Thus, the exposure comes before the disease. These studies also compare groups with lower and higher levels of exposure.

All epidemiological studies need to identify and take into account *confounding factors*, i.e. those that can affect both exposure and outcome. A common example is age: getting older increases the risk of many diseases, but being older can also mean more exposure. Statistical analyses can take into account confounding factors to refine the effect of the exposure of interest.

Epidemiological studies often report the risk of a disease in one group compared to the risk in another group, thus a *relative* measure. They may have different names - relative risk, hazard ratio, odds ratio - but basically mean the same thing. In all our registry studies, we report disease risk in this way. For comparison, we have sometimes used the entire population of Blekinge except Ronneby. In other studies, we have compared residents of Ronneby who have never been registered at an address with contaminated water with persons who have lived at an address with contaminated water for a longer or shorter time.



To translate a relative risk measure into a description of the actual risk at the individual level, one also needs to know something about how common the disease in question is. A 20% increase in risk for a very rare disease means that the disease, although slightly more common, will still be very rare. On the other hand, a 20% increase in risk for a common disease means that the disease will be even more common.

In epidemiological studies, the aim is not only to report the risk but also to give an indication of the precision or uncertainty of the measure. A common approach is to provide a *confidence interval*, a range of plausible values for the risk, reflecting statistical uncertainty. In simple terms, if the confidence interval is very large, it means that the risk estimate is very uncertain, but if the interval is small, the estimate is more reliable.

In the epidemiological studies presented on the following pages, we have tried as far as possible to identify groups with clearly contrasting exposure levels to find the best measures of the outcome we are studying, and to take relevant confounding factors into account. The reported risk measures describe what happened in the groups studied, while the confidence intervals indicate the precision in our estimates. Even in a study with many participants, the uncertainty in the risk estimate will be large for rare conditions, while the risk estimate will be more confident for common conditions. This is simply because the more data available, the more confidence in the result of the analysis.

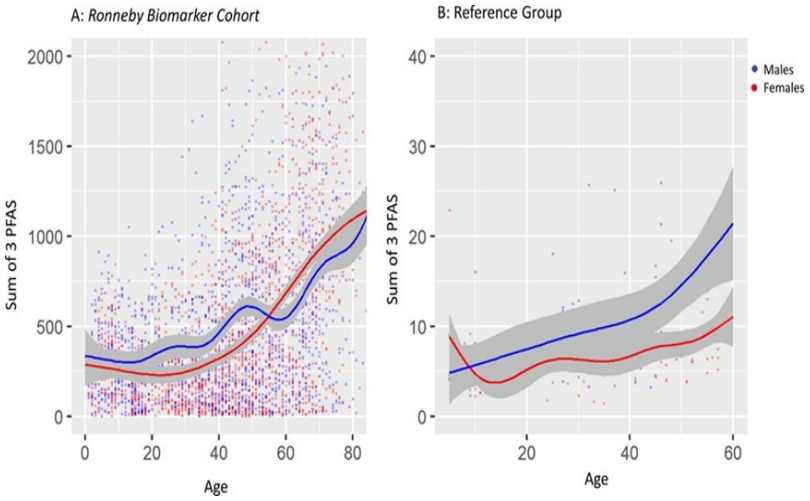
It is also important in any interpretation of the findings of a single study to relate the findings to other epidemiological investigations, experimental studies, and other information. If several studies produce similar results, this strengthens the evidence for a relationship between exposure and outcome. In epidemiological research, an increase in risk is usually required to be shown in more than one study before conclusions about associations can be drawn.

Summary of scientific publications

Exposure levels

Xu Y, Nielsen C, Li Y, Hammarstrand S, Andersson EM, Li H, Olsson DS, Engström K, Pineda D, Lindh CH, Fletcher T, Jakobsson K. Serum perfluoroalkyl substances in residents following long-term drinking water contamination from firefighting foam in Ronneby, Sweden. Environ Int. 2021 Feb;147:106333. doi: 10.1016/j.envint.2020.106333.

This study provides a good picture of the exposure levels to PFAS substances in terms of blood concentrations for Ronneby residents who participated in an open sampling programme in 2014-2015. About 13% of the municipality's residents participated. For comparison, a group from the neighboring municipality Karlshamn with only background exposure was also examined.



The figure shows the summarised content of the three PFAS compounds in serum (ng/ml) at different ages among participants from Ronneby (A) and Karlshamn (B). Each individual is shown as a dot. The lines show average values. Data for men and women are shown separately

Note that the scales on the y-axis are very different.

The main findings of the study

- Very high PFAS levels were observed among those who had contaminated water at home.
- Many participants from Ronneby had elevated PFAS levels even though they had never lived in areas with contaminated drinking water.
- PFAS levels increased with age.
- Women of childbearing age had lower PFAS levels than men in the same age category.
- From an international perspective, the average levels of PFOS and PFHxS are very high.

	PFHxS (ng/ml)	PFOS (ng/ml)	PFOA (ng/ml)
Ronneby (n=3293)	114	135	4,5
Karlshamn (n=219)	0,84	3,9	1,5
International comparison			
C8,USA	3,3	19	33
Veneto, Italy	3,0	8,7	14

The geometrical mean in the investigated population is given in the Table.

PFAS levels after cessation of exposure to contaminated drinking water

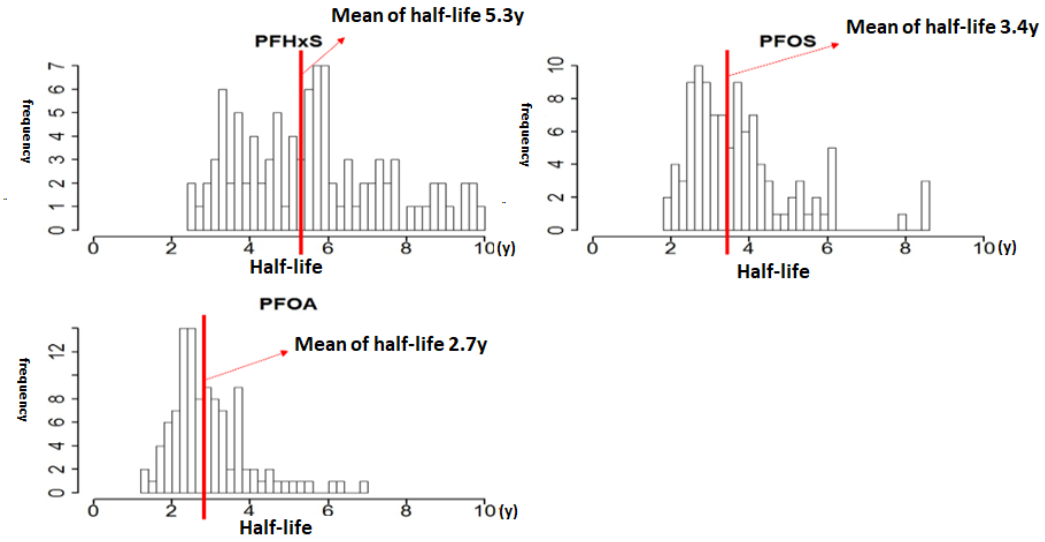
Li, Y., Fletcher, T., Mucs, D., Scott, K., Lindh, C. H., Tallving, P., & Jakobsson, K. (2018). Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occup Environ Med . 2018 Jan;75(1):46-51. doi: 10.1136/oemed-2017-104651

Xu Y, Fletcher T, Pineda D, Lindh CH, Nilsson C, Glynn A, Vogs C, Norström K, Lilja K, Jakobsson K, Li Y. Serum Half-Lives for Short- and Long-Chain Perfluoroalkyl Acids after Ceasing Exposure from Drinking Water Contaminated by Firefighting Foam. Environ Health Perspect. 2020 Jul;128(7):77004. doi:10.1289/EHP6785. Epub 2020 Jul 10. PMID: 32648786; PMCID: PMC7351026.

Li Y, Andersson A, Xu Y, Pineda D, Nilsson CA, Lindh CH, Jakobsson K, Fletcher T. Determinants of serum half-lives for linear and branched perfluoroalkyl substances after long-term high exposure-A study in Ronneby, Sweden. Environ Int. 2022 May;163:107198. doi: 10.1016/j.envint.2022.107198.

PFAS compounds cannot be broken down in the body; they are excreted unchanged in urine and faeces. The rate of excretion differs between different PFAS compounds and between individuals. We are therefore investigating how excretion occurs in a range of studies, and trying to understand why there are differences.

We have followed 110 persons of all ages from Ronneby, providing up to 10 blood samples per person during the period 2014-2018. The half-life (the time it takes for blood levels to fall to half the initial value) of the dominant PFAS compounds after the end of exposure to contaminated drinking water is shown in the figure below. A similar study from another part of Sweden shows the excretion of a range of other PFAS compounds.



The main findings of the three studies

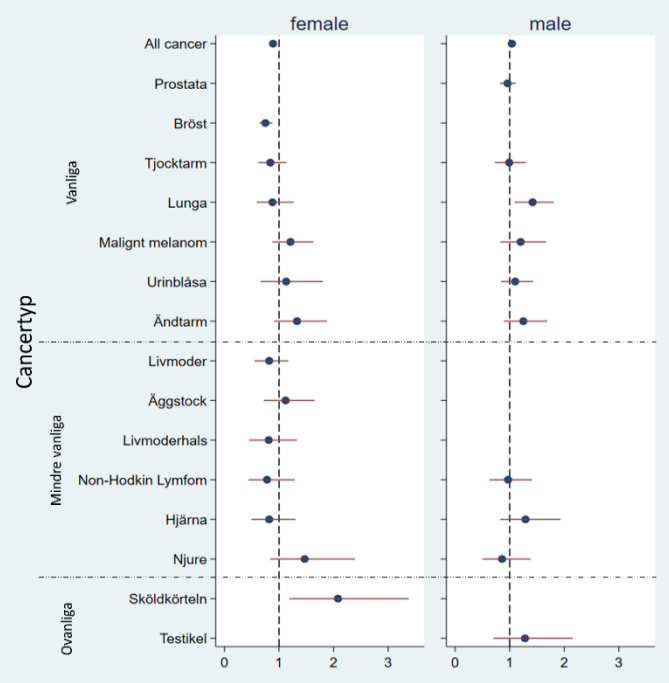
- The half-life of the PFAS compounds that dominated the contaminated drinking water is on average several years, with large variations between individuals.
- For women of childbearing age, blood levels drop more rapidly, as PFASs also leave the body during menstruation, childbirth, and breastfeeding.
- Younger persons excrete PFAS slightly faster than older persons.
- Persons with good kidney function excrete PFAS faster than those with reduced kidney function.
- PFAS compounds with short carbon chains are excreted faster than compounds with long carbon chains.

Register studies - disease diagnoses, all ages

Cancer

Li H, Hammarstrand S, Midberg B, Xu Y, Li Y, Olsson DS, Fletcher T, Jakobsson K, Andersson EM. Cancer incidence in a Swedish cohort with high exposure to perfluoroalkyl substances in drinking water. Environ Res. 2022 Mar;204(PtC):112217. doi: 10.1016/j.envres.2021.112217.

Data on 35 different cancer diagnoses during the period 1985-2016 were retrieved from the Cancer Registry for over 60,000 persons, who were registered in Ronneby at some point in 1985-2013. Their PFAS exposure was based on annual address and drinking water distribution data. Comparisons of cancer incidence among men and women were made in a first step against other Blekinge residents (see figure).



Cancer incidence (standardised with respect to age and gender) among Ronneby residents who have ever lived at an address with PFAS-contaminated drinking water, the comparison group is everyone in Blekinge except Ronneby. The figure shows observed risk (dot) with confidence interval (red line).

A reduced risk is indicated to the left of the dashed line.

An increased risk is indicated to the right of the dashed line.

A horizontal line crossing the dashed line means that there was no statistically significant difference compared to others in Blekinge.

In-depth analyses considered how long and during what time the persons had been registered in Ronneby with contaminated water in their homes. Furthermore, age, gender and highest level of education were used as markers of sociodemographic conditions, which may affect the risk of certain cancers.

The main findings of the study

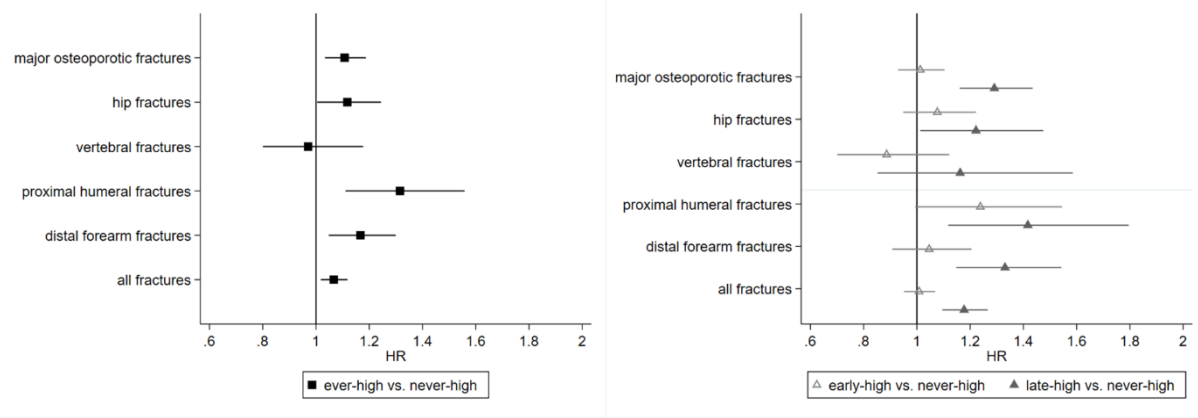
- *Common cancers*: There was no increased risk of all cancers combined, nor was there an increased risk of the most common cancers (prostate and breast cancer), among those who had ever lived at an address with contaminated drinking water. The in-depth analyses showed the same pattern. An increased risk of lung cancer among men is most likely explained by an increased prevalence of smokers in the high- exposure category, and the in-depth analyses did not show any increase in risk related to PFAS exposure.
- *Less common cancers*: The in-depth analyses showed an increased risk of kidney cancer among those with the highest PFAS exposure.
- *Unusual cancers:* There were also indications of an increased risk of testicular cancer, possibly also thyroid cancer in women. However, a small number of cases makes the observations more uncertain.

Osteoporosis fractures

Xu Y, Hansson E, Andersson EM, Jakobsson K, Li H. High exposure to perfluoroalkyl substances in drinking water is associated with increased risk of osteoporotic fractures-A cohort study from Ronneby, Sweden. Environ Res. 2022. DOI: 10.1016/j.envres.2022.114796.

PFAS can accumulate in bone tissue. Several studies in population groups with background exposure have indicated a risk of reduced bone density (osteoporosis), but knowledge is still very limited and there is a lack of data from highly exposed groups. Reduced bone density means an increased risk of fracture. As osteoporosis is common among the elderly in Sweden, it was important to investigate the risk of osteoporosis fractures in Ronneby, where many residents have had long-term and high exposure to PFAS.

Data on the diagnosis of osteoporosis-related fractures (vertebrae, upper arm, forearm/wrist and hip) were obtained from the national patient register. The study included over 63,000 persons who were registered in the municipality at some point during the period 1985-2013. Their residential addresses were used to classify exposure to PFAS-contaminated water: never (never-high, comparison group), ever (ever-high), only during an early period 1985-2004 (early-high) or a late period 2005-2013 (late-high). Many in the latter group had also been exposed in the earlier period.



The figure shows the fracture risk expressed as Hazard Ratio (HR). The different exposure groups are compared to those who have never lived at an address with contaminated drinking water. Horizontal lines indicate the confidence interval or uncertainty in the risk estimate. A horizontal line that is entirely to the right of the vertical line means that the difference from the comparison group is statistically significant. HR=1 indicates the same risk as in the comparison group. HR=2 means a doubled risk.

The main findings of the study

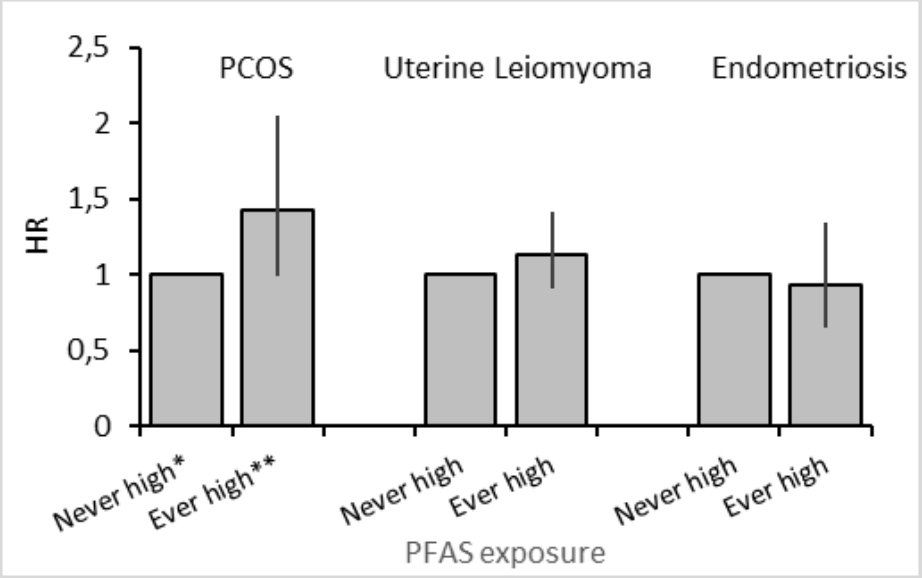
- The risk of osteoporosis-related fractures was increased in the group with the highest PFAS exposure.
- There was no clear gender difference.
- Our findings reinforce earlier suspicions that PFASs can adversely affect bone tissue, thereby increasing the risk of fractures.

Gynaecological diseases

Hammarstrand S, Jakobsson K, Andersson E, Xu Y, Li Y, Olovsson M, Andersson EM. Perfluoroalkyl substances (PFAS) in drinking water and risk for polycystic ovarian syndrome, uterine leiomyoma, and endometriosis: A Swedish cohort study. Environ Int. 2021 Aug 12;157:106819. doi: 10.1016/j.envint.2021.106819.

Several persistent environmental pollutants have been shown to affect the reproductive hormone system in women, including PFAS. We have therefore investigated the prevalence of the gynaecological diseases including polycystic ovarian syndrome (PCOS, ovarian cysts and elevated testosterone levels), uterine leiomyoma (muscle nodules in the uterus) and endometriosis (growth of endometrium outside the uterus).

We studied disease diagnoses during the period 1987-2013 among about 29,000 women who lived in Ronneby anytime between 1985 and 2013. The women were divided into different exposure categories: *never* lived at an address with heavily PFAS-contaminated drinking water (comparison group); *ever* lived at an address with PFAS-contaminated drinking water; *early high exposure*, anytime between 1985-2004; and *late high* exposure, anytime between 2005-2013.



The figure shows the disease risk for women aged 20-50 years expressed as Hazard Ratio (HR). The vertical line indicates the uncertainty in the risk estimate. HR=1 indicates the same risk as in the comparison group. HR=2 means a doubled risk.

The main findings of the study

- Women aged 20 to 50 years, who had lived at addresses with contaminated drinking water, had an increased risk of PCOS compared to those who had never lived at addresses with contaminated drinking water. The highest risk estimate was observed in the group with late high exposure.
- For endometriosis, no increased risk was seen.
- For uterine leiomyoma, an increased risk was suggested in women before age 50, but when older women were also included in the analyses, this finding disappeared.

Pregnancy complications

Ebel M, Rylander L, Fletcher T, Jakobsson K, Nielsen C. Gestational hypertension, preeclampsia, and gestational diabetes mellitus after high exposure to perfluoroalkyl substances from drinking water in Ronneby, Sweden. Environ Res. 2023 Oct 5;239(Pt 1):117316. doi: 10.1016/j.envres.2023.117316.

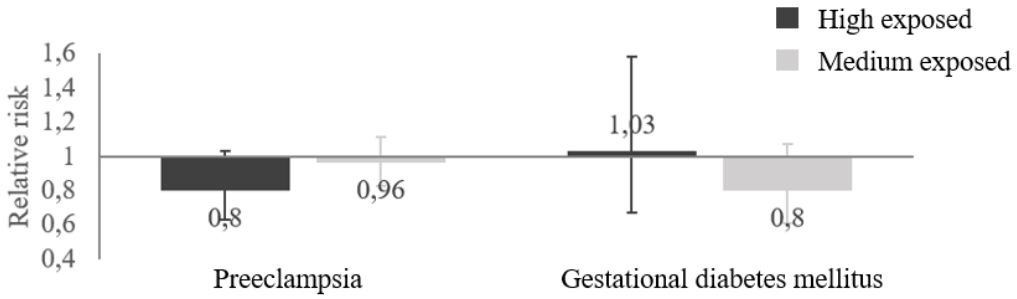
Pregnancy complications such as pre-eclampsia/high blood pressure and gestational diabetes can pose risks to mother and child, both during and after pregnancy. Some previous studies have linked PFAS to an increased risk of developing pregnancy complications, but the association has never been studied after high exposure to PFOS and PFHxS.

We identified women who were registered in Blekinge sometime between 1990 and 2013, and who gave birth in 1995-2013 (27,292 pregnancies). We analysed diagnostic data from the Medical Birth Register. This also made it possible to simultaneously take into account other factors that affect the risk of pregnancy complications such as age, smoking habits, country of birth, level of education and number of previous pregnancies. The women's PFAS exposure was categorised into three groups:

- Background exposure (comparison group): Women who have lived in Blekinge but never in Ronneby during five years before giving birth.

  Medium exposure: Women living in Ronneby, but never with contaminated water at their home address during five years before giving birth

- High exposure: Women living in Ronneby, with contaminated water at their home address at some point during five years before giving birth



The figure shows the disease risk expressed as relative risk (RR). The different exposure groups are compared to those who have never lived at an address with contaminated drinking water. The vertical line indicates the confidence interval (uncertainty in the risk estimate). RR=1 indicates the same risk as in the risk comparison group. RR less than 1 means a lower risk than in the comparison group.

The main findings of the study

- We found no increased risk of gestational diabetes, neither in the high-exposure group nor in the intermediate group, compared to the other women from the Blekinge region.
- We found no increased risk of high blood pressure or preeclampsia, neither in the high-exposure group nor the intermediate group, compared to other women from Blekinge.
- The study included a large cohort with highly variable PFAS exposure and is based on data from the Swedish maternity services, which reach almost all women. It is therefore a reliable result.

Combined studies (diagnostic register, drug prescription, biomarkers)

Thyroid disease

Andersson EM, Scott K, Xu Y, Li Y, Olsson DS, Fletcher T, Jakobsson K. High exposure to perfluorinated compounds in drinking water and thyroid disease. A cohort study from Ronneby, Sweden. Environ Res 2019,176:108540. Doi 10.10167j.environres.2019.108540

Li Y, Xu Y, Fletcher T, Scott K, Nielsen C, Pineda D, Lindh CH, Olsson DS, Andersson EM, Jakobsson K. Associations between perfluoroalkyl substances and thyroid hormones after high exposure through drinking water. Environ Res. 2021 Mar;194:110647. doi: 10.1016/j.envres.2020.110647.

The thyroid gland is one of the most important hormone-producing organs in the body. Its various hormones affect almost all body functions by regulating metabolism, i.e. how the body converts food into energy. Both overproduction and underproduction can lead to ill health. During the foetal period, the mother's thyroid hormones are needed for the development and growth of the foetus.

Experimental animal studies have shown that PFASs can adversely affect hormonal balance, but a large number of epidemiological studies have given very different results. Almost all these studies have been conducted at background exposure. Therefore, it was urgent to investigate a highly exposed group.

We examined the relationship between PFAS exposure and thyroid disease in three different ways.

i. A register study covering more than 63,000 persons who have lived in Ronneby at some point. Data on disease diagnoses and drug use were obtained from national healthcare registers.
ii. A biomarker study investigating the relationship between thyroid hormones and PFAS levels in the blood among 3397 persons from Ronneby and 226 from Karlshamn (representing background exposure levels).
iii. A study of the prescribing pattern for drugs used in thyroid disease at the different medical centres in Ronneby and Karlshamn during the period 2009-2016.

PFAS exposure was estimated by measured PFAS levels (the hormone level studies), and by address-based classification of drinking water occurrence in the home (diagnoses, drug use). The statistical analyses examined possible associations in different age groups and for women and men separately. The results could vary slightly between different subgroups. We present the overall picture here.

Key findings of the studies

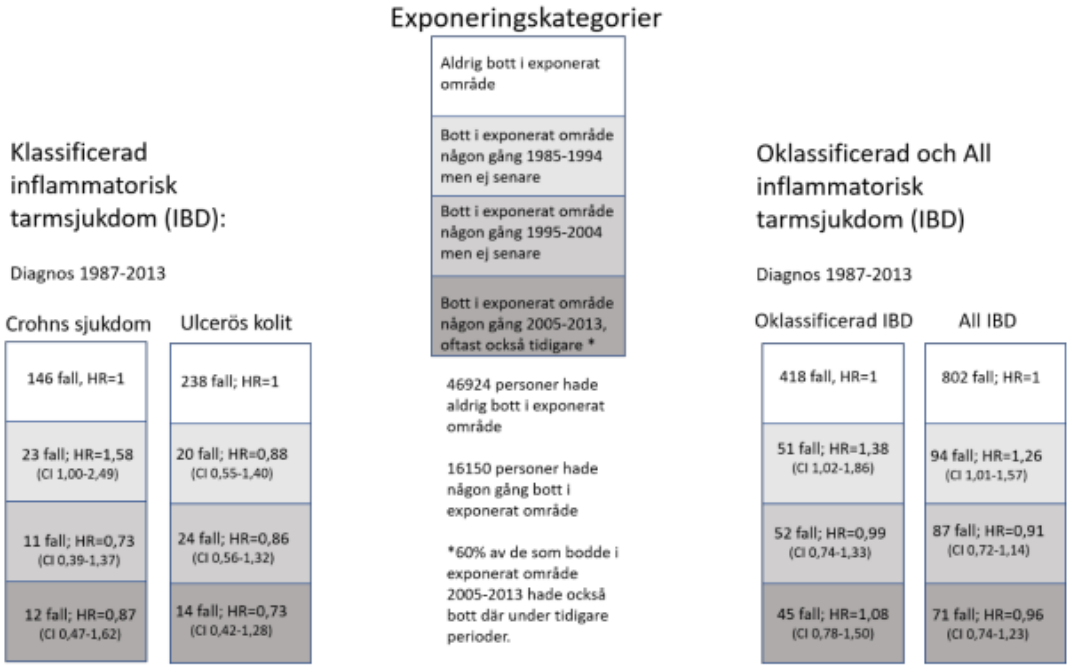
- We did not find an increased risk of thyroid disease among those with the highest exposure to PFAS in drinking water, either among women or men.
- Among middle-aged and elderly, we did not see any correlation between the levels of the different thyroid hormones in the blood and the levels of PFAS in the blood.
- Among children and young persons, the results were difficult to interpret - there were no consistent findings in either direction.
- The health centre in the highly contaminated water district (Kallinge) did not have a higher prescription of medicines for thyroid disease than the other health centres in Ronneby or in Karlshamn.
- The overall result of the three different studies is similar: we have not been able to demonstrate any negative impact on thyroid function.

Inflammatory bowel disease (IBD)

Xu Y, Li Y, Scott K, Lindh CH, Jakobsson K, Fletcher T, Ohlsson B, Andersson EM. Inflammatory bowel disease and biomarkers of gut inflammation and permeability in a community with high exposure to perfluoroalkyl substances through drinking water. Journal: Environ Res. 2020 Feb;181:108923. doi: 10.1016/j.envres.2019.108923.

There are suspicions that PFASs entering the gastrointestinal tract could affect the gut lining and ultimately lead to inflammatory bowel disease.

To investigate whether the PFAS-contaminated drinking water has led to an increased incidence of inflammatory bowel disease in Ronneby, a large register study was carried out, which included over 63,000 persons who had been registered in the municipality at some point during the period 1985-2013. Data on diagnoses of inflammatory bowel disease (ulcerative colitis, Crohn's disease and unspecified colitis) were obtained from the national patient register.



The disease risk is expressed as a hazard ratio (HR), in relation to the observed risk among those who have ever lived in Ronneby, but never had contaminated water in their homes. HR 1.0 thus means no increased risk. The confidence interval (CI) expresses the uncertainty in the risk estimate.

In addition, the presence of the biomarkers calprotectin, often used in the diagnosis of inflammatory bowel disease, and zonulin, a marker of permeability of the intestinal wall used in research, was measured in the faeces from 189 persons. The aim was to investigate whether there was an association between PFAS exposure and signs of inflammation in the intestinal mucosa.

The main findings of the study

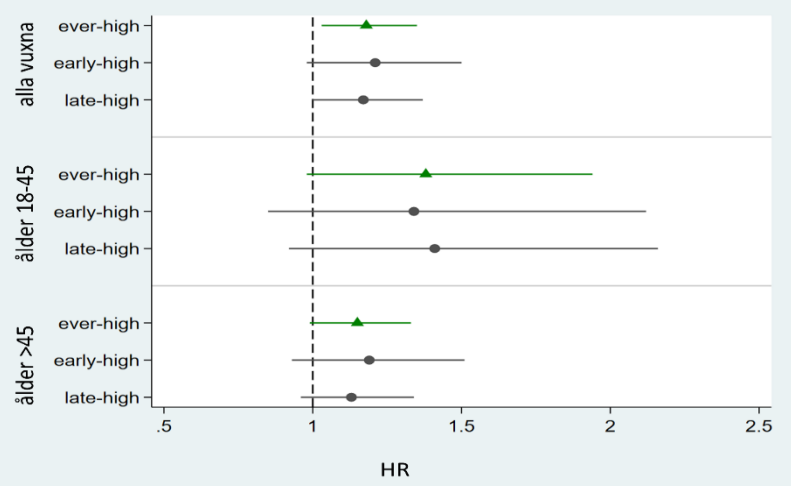
- The registry study showed no association between exposure to PFAS in drinking water and an increased risk of inflammatory bowel disease.
- The biomarker study also showed no association between PFAS levels in the blood and markers of inflammation in the intestinal mucosa.

Type 2 diabetes

Xu Y, Jakobsson K, Harari F, Andersson EM, Li Y. Exposure to high levels of PFAS through drinking water is associated with increased risk of type 2 diabetes-findings from a register-based study in Ronneby, Sweden. Environ Res. 2023 May 15;225:115525. doi: 10.1016/j.envres.2023.115525.

PFAS substances have been suspected of increasing the risk of metabolic diseases, including type 2 diabetes. However, epidemiological studies examining large groups of persons with background levels of PFAS exposure have produced contradictory results.

We followed more than 55,000 adults who ever were registered in Ronneby during the period 1985-2013. Information on diagnosed type 2 diabetes was obtained from the national patient register and the pharmaceutical register. The persons' residential addresses were used to classify their exposure to PFAS-contaminated water: never, ever, only during an early period 1985-2004 (early-high) or a late period 2005-2013 (late-high). We also examined the risk of diabetes in different age groups (under or over 45 years old).



The figure shows the risk of diabetes expressed as Hazard Ratio (HR). The different exposure groups are compared with those who have never lived at an address with contaminated drinking water.

Horizontal lines indicate uncertainty in the risk estimate. A horizontal line that is entirely to the right of the vertical line (increased risk) means that the difference with the comparison group is statistically significant.

HR=1 indicates no increased risk. HR=2 means a doubled risk.

In addition, the study compared prescription patterns for diabetes drugs during the period 2006-2022 from health centres in Kallinge (highly contaminated drinking water), the rest of Ronneby municipality, and the rest of Blekinge.

The main findings of the study

- We found an increased risk of type 2 diabetes among those with the highest exposure to PFAS.
- The increased risk was most evident at younger ages.
- The prescription of medicines for the treatment of type 2 diabetes was slightly more common in the area with heavily contaminated drinking water than in the other health centres.
- Our findings reinforce the suspicion that there may be a causal link between PFAS exposure and diabetes, but more research is needed.

Register and medical record studies – Children

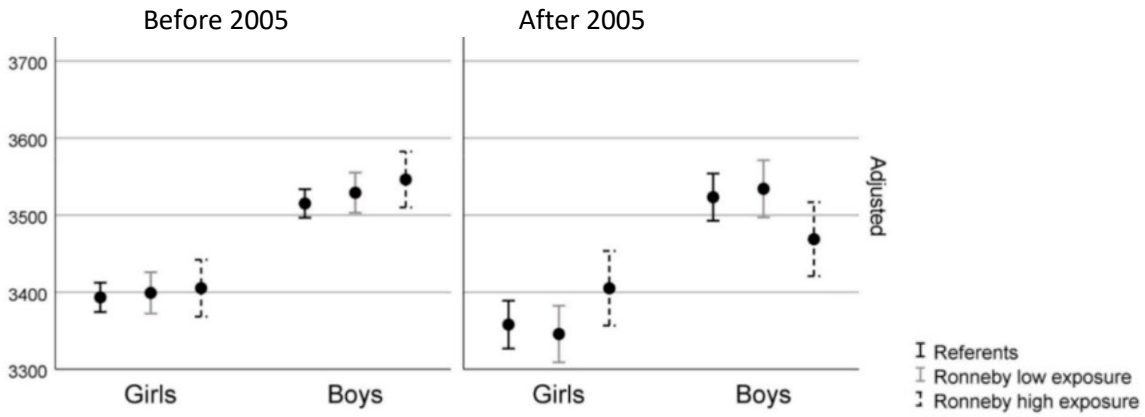
Birth weight

Engström K, Axmon A, Nielsen C, Rignell-Hydbom A. High *in utero* exposure to perfluoroalkyl substances from drinking water and birth weight: A cohort study among infants in Ronneby, Sweden. Int J Environ Res Public Health. 2022 Feb 18;19(4):2385. doi: 10.3390/ijerph19042385. PMID: 35206572.

PFASs are transferred from the mother to the foetus during pregnancy. Several studies have found that birth weight is slightly lower with increased levels of PFASs in the mother's blood, but these studies have only been conducted at low exposure levels.

We identified all children born in Blekinge between 1995 and 2013 in the Medical Birth Register and collected register data on birth weight, length of pregnancy and confounding factors such as the child's sibling order, the mother's level of education and smoking.

PFAS exposure was determined according to where the mother lived during the five years before the birth; in Ronneby with contaminated water (high exposure, n=823), in Ronneby without contaminated water (low exposure, n=3,452) or in the rest of the county (n=9,692). Since PFAS levels were assumed to have increased over time we distinguished between children born before and after 2005.



Mean birth weight with 95% confidence intervals stratified for infant sex and period (before or after 2005). Adjusted models include gestational age, parity, and maternal age, civil status, smoking habits, and BMI in early pregnancy.

The main findings of the study

- Boys born after 2005 weighed on average 54 g less than boys from the rest of Blekinge if the mother had contaminated water in the home.
- Girls born after 2005 weighed on average 47 g more than girls from the rest of Blekinge if the mother had contaminated water in the home.
- Before 2005, there were no differences in birth weight between Ronneby children and children from the rest of Blekinge.
- After 2005 there was no difference in birth weight for children of mothers living in Ronneby without polluted water compared to children from the rest of Blekinge.
- The observed effect of PFAS exposure on birth weight is much smaller than previously observed in studies among women with background exposure.

Breastfeeding

Nielsen C, Li Y, Lewandowski M, Fletcher T, Jakobsson K. Breastfeeding initiation and duration after high exposure to perfluoroalkyl substances through contaminated drinking water: A cohort study from Ronneby, Sweden. Environ Res. 2022 May 1;207:112206. doi: 10.1016/j.envres.2021.112206.

Breastfeeding has positive health benefits for both mother and baby. Effective breastfeeding depends on both complex social factors and complex hormonal processes. As PFASs are endocrine disruptors, there is a suspicion that exposure could interfere with the development of the mammary glands and ultimately the mother's ability to produce enough breast milk. Animal studies have supported this suspicion, and some studies of women with background exposure to PFASs point in the same direction. However, it is not known how breastfeeding is affected by high PFAS exposure.

Our study is based on information about breastfeeding from the BVC records of 2079 children born in Ronneby municipality between 1999 and 2009. We also collected BVC records from a comparison group of 295 children from Karlshamn. Information on other important factors that may affect breastfeeding was also obtained from the BVC records and was used to make adjusted risk estimates. Women's PFAS exposure was based on residential addresses and water distribution data.

- ○ Background exposure (comparison group): Women who have lived in Blekinge but never in Ronneby during five years before giving birth.
- ○ Medium exposure: Women living in Ronneby, but never with contaminated water at their home address during five years before giving birth.
- ● High exposure: Women living in Ronneby, with contaminated water at their home address at some point during five years before giving birth.

The main findings of the study

- It was more common among highly exposed mothers in Ronneby to fail to establish effective breastfeeding than in the comparison group from Karlshamn, 5% compared to 2%.
- A lower proportion of highly exposed first-time mothers were breastfeeding at six months, 54%, compared to 65% in Karlshamn (they were 1.6 times more likely have stopped breastfeeding at 6 months, adjusted for confounding factors). They were also more likely to have started complementary feeding at three months of age.
- There was no increased risk of a shortened breastfeeding period among multiparous women.

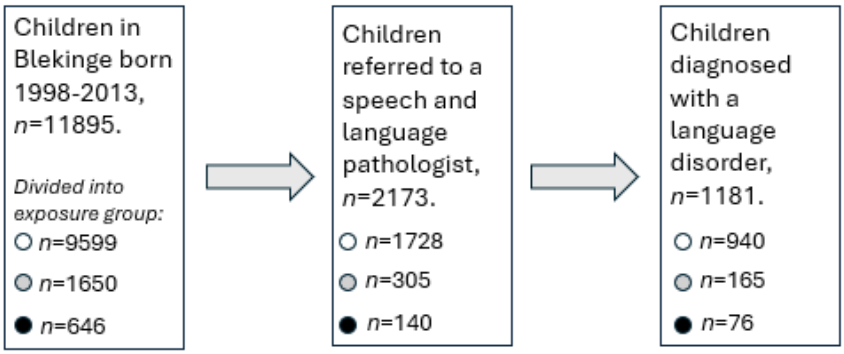
Language development

Stübner, C., Martinsson, M., Jakobsson, K., Gillberg, C., Nielsen, C., & Miniscalco, C. (2022). Developmental language disorders in preschool children after high exposure to perfluoroalkyl substances from contaminated drinking water in Ronneby, Sweden. Environ Epidemiol. 2022 Dec 14;7(1):e233. doi: 10.1097/EE9.0000000000000233.

Fetal exposure to PFASs is suspected to adversely affect brain development, but results from epidemiological studies are uncertain and studies from highly exposed populations are lacking. Language ability is a good marker of brain development, and all children in Sweden have long been examined at the child health services in a standardised way. If a language disorder is suspected, children are referred to a speech therapist for further assessment.

The study includes all children in Blekinge born between 1998 and 2013.

- ○ Background exposure: Mother living in Blekinge but never in Ronneby during five years before giving birth.
- ◌ Medium exposure: Mother living in Ronneby, but never with contaminated water at their home address during five years before giving birth.
- ● High exposure: Mother living in Ronneby, with contaminated water at their home address at some point during five years before giving birth.



We calculated the risk of referral to a speech and language pathologist after screening at the child healthcare center and the risk of a language disorder diagnosis after at least two clinical assessments by a speech and language pathologist. The analyses were adjusted for the mother's age, level of education and smoking as well as the child's gender and sibling order. These factors are of great importance for children's language development.

The main findings of the study

- Highly exposed children in Ronneby had an increased risk of being referred to a speech therapist compared to the background group.
- Highly exposed girls (but not boys) had an increased risk of being diagnosed with a language disorder.
- The medium exposure group in Ronneby had no increased risk of referral or diagnosis compared to children from the rest of Blekinge.
- This is the first study showing increased risk of developmental language disorders in association to prenatal exposure to high levels of PFOS and PFHxS, and more studies are needed.

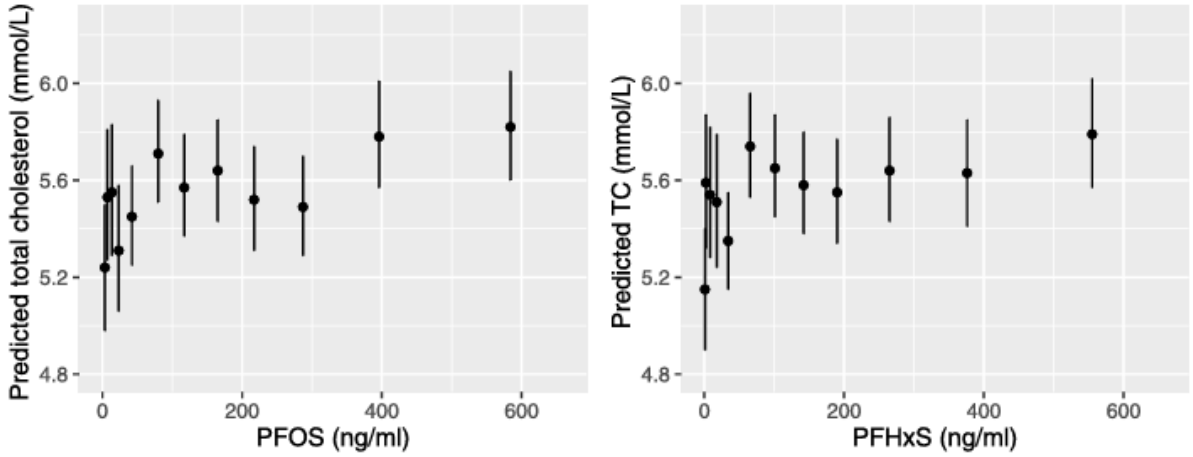
Biomarker studies - all ages

Blood lipids

Li Y, Barregard L, Xu Y, Scott K, Pineda D, Lindh CH, Jakobsson K, Fletcher T. Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. Environ Health. 2020 Mar 14;19(1):33. doi: 10.1186/s12940-020-00588-9.

An association between exposure to PFAS and elevated levels of blood lipids, especially cholesterol, has been observed in many epidemiological studies, mainly in the general population with background exposure. It was therefore urgent to investigate the situation in Ronneby, where many persons have had much higher PFAS exposure via drinking water for a long time.

1,815 persons aged 20-60 years who participated in the open sampling in Ronneby in 2014-16 and 130 persons from Karlshamn (control area without PFAS-contaminated drinking water) were included in the study. The associations between PFAS levels and blood lipids were calculated adjusting for age, gender and BMI, which also affect blood lipid levels.



Adjusted means for total cholesterol in PFAS ventile (up to the 20th percentile) and deciles. The means were adjusted with age, sex, and BMI in quartiles.

The main findings of the study

- At the group level, 7-9% increases in total cholesterol and LDL (the "bad" cholesterol) were seen among participants from Ronneby compared to participants from Karlshamn.
- The risk increase appeared to level off at the highest PFAS levels. This has also been seen in studies of high exposure to PFOA.
- Our findings reinforce the evidence that there is a causal link between PFAS exposure and blood lipids, especially cholesterol.

Epigenetic markers

Xu Y, Jurkovic-Mlakar S, Li Y, Wahlberg K, Scott K, Pineda D, Lindh CH, Jakobsson K, Engström K. Association between serum concentrations of perfluoroalkyl substances (PFAS) and expression of serum microRNAs in a cohort highly exposed to PFAS from drinking water. Environ Int. 2020 Mar;136:105446. doi: 10.1016/j.envint.2019.105446.

Xu Y, Jurkovic-Mlakar S, Lindh CH, Scott K, Fletcher T, Jakobsson K, Engström K. Associations between serum concentrations of perfluoroalkyl substances and DNA methylation in women exposed through drinking water: A pilot study in Ronneby, Sweden. Environ Int. 2020 Dec;145:106148. doi: 10.1016/j.envint.2020.106148. Epub 2020 Sep 30.

Xu Y, Lindh CH, Fletcher T, Jakobsson K, Engström K. Perfluoroalkyl substances influence DNA methylation in school-age children highly exposed through drinking water contaminated from firefighting foam: a cohort study in Ronneby, Sweden. Environ Epigenet. 2022 Feb 4;8(1):dvac004. doi: 10.1093/eep/dvac004.

Surprisingly little is still known about the biological mechanisms by which exposure to PFAS can lead to adverse effects. Some studies, mostly in animals, have shown that changes in so-called epigenetic processes in cells are a particular mechanism. Epigenetics refers to genetic changes, which are not due to changes in the DNA sequence, but instead affect how the information in the DNA sequence is utilised in the cell, e.g. in the synthesis of proteins.

Epigenetic markers can indicate cellular damage mechanisms. We have therefore examined the pattern of a large number of epigenetic markers, so-called microRNAs, among 293 women from Ronneby and Karlshamn. We then examined another epigenetic marker, DNA methylation, in 59 women aged 20-47 years and in 63 school-age children. For some of the many markers studied, we found higher levels among those with the highest PFAS exposure. However, our studies are small and much larger studies are needed to verify these findings.

The main findings of the studies

- Our results indicate that PFAS exposure may be associated with changes in epigenetic markers, which in turn are associated with some specific genes or specific functions.
- This is of interest in terms of understanding what happens at the cellular level, but much research remains to be done to understand what this means in terms of health and disease.

COVID-19 studies

COVID-19 infections in Ronneby during the first wave

Nielsen C, Jöud A. Susceptibility to COVID-19 after high exposure to perfluoroalkyl substances from contaminated drinking water: an ecological study from Ronneby, Sweden. Int J Environ Res Public Health. 2021; 18(20):10702. https://doi.org/10.3390/ijerph182010702.

At the height of the pandemic, there were concerns that PFASs could increase the risk of COVID-19, as PFASs have been linked to adverse effects on the immune system. It was therefore important to be able to answer whether there was an increased risk in population groups exposed to high PFAS levels, and two studies addressed this question. The first one was an ecological study, where information is available at the group level but not for the individuals. The neighbouring municipality Karlshamn, where the drinking water was not contaminated with PFASs, was used for comparison.

From the Swedish Public Health Agency's register of infectious diseases, we received information on all PCR-confirmed cases of COVID-19 among persons over 18 years of age from Ronneby and Karlshamn until March 2021. We found 68 cases per 1000 inhabitants in Ronneby and 56 cases per 1000 inhabitants in Karlshamn. Taking differences in the structure of the population in terms of gender and age into account, we were able to calculate that in the initial stage, 19% more cases of COVID-19 occurred in Ronneby than in Karlshamn.

An extended follow up was summarised in the study on vaccination efficiency (see page 30). The data presented as unadjusted rates confirmed the higher incidence of COVID-19 cases in Ronneby compared to Karlshamm in the first wave, but this excess was not evident in comparison with the other nonexposed municipalities in Blekinge County, nor in subsequent COVID-19 waves[9].

The main findings

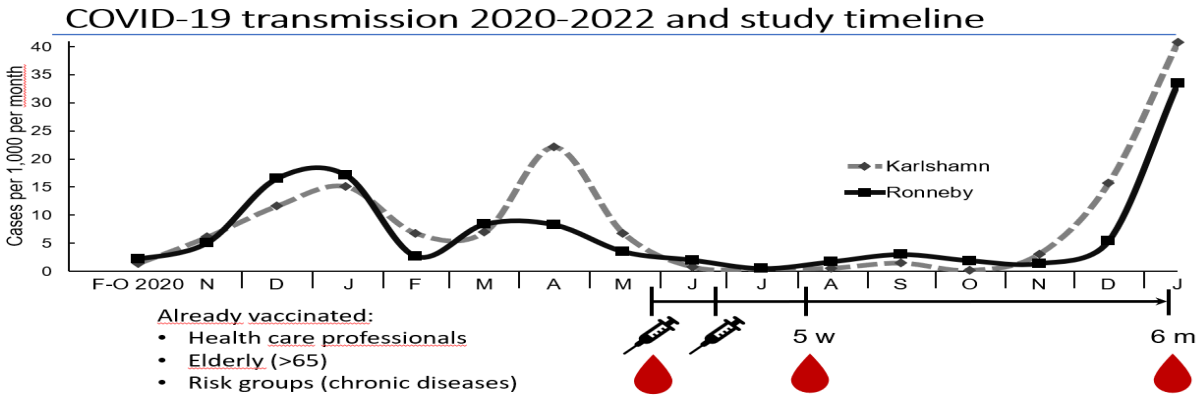
- COVID-19 incidence was higher in Ronneby than in the comparative town of Karlshamn during the first wave.
- Over a longer period, there was not an indication of a higher rate in the PFAS affected area compared to other areas of Blekinge county.
- Since the spread of infection occurs in waves and is changeable over time and space, it is necessary to study a longer time course and include the total Blekinge population in studies with individual level information on age and other risk factors. Such a cohort study is under way.

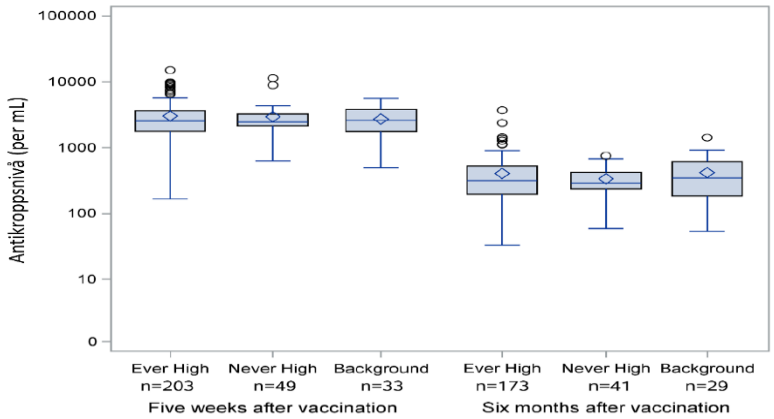
[9] Andersson AG et al. Environ Health Perspect. 2023 Aug;131(8):87007. doi:10.1289/EHP11847

Antibody formation after COVID-19 vaccination

Andersson AG, Lundgren A, Xu Y, Nielsen C, Lindh CH, Pineda D, Cederlund J, Pataridou E, Søgaard Tøttenborg S, Ugelvig Petersen K, Fletcher T, Lagging M, Bemark M, Jakobsson K, Li Y. High Exposure to Perfluoroalkyl Substances and Antibody Responses to SARS-CoV-2 mRNA Vaccine-an Observational Study in Adults from Ronneby, Sweden. Environ Health Perspect. 2023 Aug;131(8):87007. doi: 10.1289/EHP11847. Epub 2023 Aug 14. PMID: 37578904; PMCID: PMC10424820.

PFASs have been shown in animal studies to affect the immune system negatively, and observations of reduced antibody levels after childhood vaccinations have formed the basis for developing guidelines for maximum permitted levels of PFASs in drinking water. Data from adults have been sparse and difficult to interpret. The COVID-19 pandemic - a new disease and a new vaccine - provided a unique opportunity to conduct a vaccine study among adults. During the summer of 2021, 319 persons from Ronneby and 48 persons from Karlshamn with only background exposure to PFAS participated in a study of vaccination responses after two doses of an mRNA vaccine.





The antibody response and cellular immune response was measured after 5 weeks and after 6 months. The figure shows the antibody levels resulting from participants who were not ill with COVID-19 before or during the study.

The main findings of the study

- All participants obtained a good vaccination response.
- We saw no differences between the exposure groups, either for antibody levels or cellular immune response. There was also no relationship between measured PFAS content and vaccination response.
- Those exposed to PFAS during early childhood did not have a reduced vaccination response compared to those exposed only in adulthood.
- We believe that the difference compared to childhood vaccine studies is explained by the maturation of the immune system in adulthood.

Studies in the Mother-Child cohort

Mother-to-child transmission of PFAS during pregnancy and breastfeeding

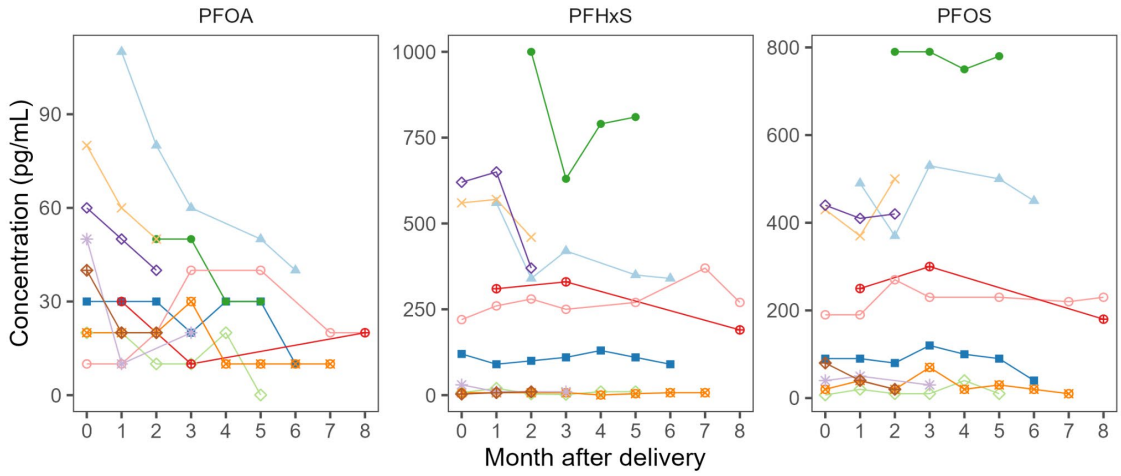
Annelise J. Blomberg, Erika Norén, Line S. Haug, Christian Lindh, Azemira Sabaredzovic, Daniela Pineda, Kristina Jakobsson,[4] Christel Nielsen[1] Transfer of perfluoroalkyl substances (PFAS) from serum to breastmilk in women highly exposed from contaminated drinking water: a study in Ronneby Mother-Child Cohort. Environ Health Perspect. 2023 Jan;131(1):17005. doi: 10.1289/EHP11292.

Blomberg AJ, Haug LS, Lindh C, Sabaredzovic A, Pineda D, Jakobsson K, Nielsen C. Changes in perfluoroalkyl substances (PFAS) concentrations in human milk over the course of lactation: A study in Ronneby mother-child cohort. Environ Res. 2022 Dec 15:115096. doi: 10.1016/j.envres.2022.115096.

Studies among background-exposed women have shown that PFASs are transferred from the mother's blood to breast milk, which can thus be an important source of exposure for infants. Knowledge of the extent of transfer in highly exposed mothers has been lacking.

The first study involved 126 mothers who provided a blood sample at birth and at least one breast milk sample early in the breastfeeding period.

The next study investigated how the PFAS content of breast milk changed over time in 74 mothers who provided both a colostrum sample and a sample of mature breast milk taken 4-12 weeks after delivery. Furthermore, 11 mothers who provided breast milk were followed throughout the breastfeeding period.



Key findings of the studies

- Mothers with higher PFAS levels in their blood also had higher PFAS levels in their breast milk.
- The level in breast milk was 1-4% of the level in the mother's blood.
- The levels of PFOS and PFHxS in breast milk did not change during the breastfeeding period, while the levels of PFOA decreased.
- There is a need to clarify the impact of PFAS exposure during breastfeeding on the child, in addition to the exposure that already occurred during pregnancy.
- The well-recognised health benefits of breastfeeding must be weighed against the risk that increased PFAS exposure may pose, so that informed recommendations on breastfeeding for highly exposed women can be made.

Ongoing and planned studies

Modelling of historical PFAS exposure

It is not possible to measure PFAS from water samples before 2013. Neither are there serum measurements in the population before that date. Therefore, we need to find another way to estimate the population's past PFAS exposure from drinking water. Some information can be obtained from hydrogeological surveys of water flow and PFAS concentrations in soil. But this is not enough for our epidemiological studies.

For all newborns, blood samples have long been preserved in the form of the so-called PKU samples, a drop of dried blood on a piece of paper. The environmental analysis laboratory at AMM in Lund can now analyse blood from PKU samples even at very low PFAS levels.

Blood from PKU samples from 288 children from Blekinge is currently being analysed. The aim is to get a picture of how the PFAS levels of newborn children and indirectly their mothers have changed during the period 1985-2013. At a later stage, we can use this to better estimate the PFAS exposure of others during different periods as we know the residential addresses and how water was distributed from the two waterworks. Our current simple exposure modelling can thus be much improved.

Follow-up in the Mother-Child cohort

We will monitor children's health and development over time in a variety of ways:

- questionnaires on the child's living conditions, physical activity, morbidity and gender development.
- information on children's growth and other health data from BVC records
- cognitive development in collaboration with a speech therapist and child psychologist.
- biological samples (blood, urine and faeces)
- routinely collected health data from student health services
- school performance at a later stage

In 2024, we will start the nine-year follow-up of the oldest children in the cohort. We will focus particularly on asthma and allergies, susceptibility to infection, and general and language development.

Other studies in children

Childhood growth

Several studies among children exposed to PFASs at background levels have shown an increased incidence of weight gain during infancy (deviation from the expected growth curve). However, there are no studies on children with high exposure to PFASs.

We have obtained information on height and weight growth from BVC records in Ronneby and Karlshamn for about 2400 children born in 1999-2009. Annual residential addresses of the mother and child provide information on PFAS exposure from drinking water.

Neuropsychiatric disabilities

Previous studies have not shown an increased risk of neuropsychiatric disabilities with PFAS exposure at background levels, but there is a lack of studies on high exposure. Since we have found an increased risk of language disorder in girls whose mothers lived in the area with contaminated drinking water, we will investigate whether there is also an increased risk of other conditions such as ADHD and autism. We are planning register-based studies in Region Blekinge's healthcare database.

Immune-related diseases

Exposure to PFAS has been related to poorer antibody response after the usual childhood vaccinations and possibly also increased susceptibility to infection in children. Previous studies have been conducted at background exposure, and there is a lack of knowledge about effects after high exposure.

We will study the entire population of Blekinge and collect data on healthcare diagnoses and drug use from regional and national registers. We will investigate whether the mother's PFAS content during pregnancy affects the child's risk of different types of immune-related conditions, namely hypersensitivity (asthma, allergy and eczema), autoimmune disease (gluten intolerance) and impaired immune system (common childhood infections such as colds, flu and ear infections).

Antibody response after childhood vaccination - a study in five Swedish childhood cohorts.

This study aims to investigate the association between PFAS exposure and antibody levels after vaccination with different types of vaccines. Biobanked serum from five Swedish child cohorts has been analysed, including serum from Ronneby children who participated in the open sampling in 2014-2015.

The national study is coordinated by Professor Anders Glynn, SLU, Uppsala.

Register studies - all ages

Cardiovascular disease

PFAS are associated with elevated cholesterol levels, which in turn is a well-known risk factor for cardiovascular disease. However, it is still not clear whether PFAS exposure leads to an increased risk of cardiovascular disease. In this study, we investigate whether there is an association between PFAS exposure and risks of hypertension, myocardial infarction and ischaemic stroke in the Ronneby cohort using registry data.

Liver impact - a combined registry and biomarker study

An association between PFAS exposure and liver enzymes and an increased risk of liver disease has been reported in several studies, mainly at background exposure. Data from highly exposed groups are lacking. We will therefore investigate this, partly as a registry study in the Ronneby cohort and partly in biobanked blood samples.

COVID-19-related studies

T-cell response after COVID-19 vaccination

This is a substudy within the investigation of the immunological response after SARS-CoV-2 vaccination (see further page 30).

COVID-19 in Blekinge

The COVID-19 pandemic brought with it a unique opportunity to study how PFAS exposure affects susceptibility to infection against a completely new disease to which the population had no immunity. We have previously described that the number of COVID-19 cases in Ronneby was elevated compared to the neighbouring municipality Karlshamn during the first year of the pandemic. We will therefore continue to study the further development of the pandemic in the county by examining the risk of PCR-confirmed COVID-19 from the Swedish Public Health Agency's register SmiNet over a longer time period. The study covers the entire population of Blekinge.

Experimental study

Excretion of PFAS

Previous studies have shown that PFASs are mainly excreted via the kidneys and the gastrointestinal tract. The slow rate of excretion is partly explained by reabsorption in the gut and the kidneys, and thus only a small amount is removed from the body.

In an ongoing study we compare the levels of a variety of PFAS compounds in serum, urine and faeces and calculate the total amount of PFASs lost per day by different routes. It appears to be significant differences between different PFAS substances, and between individuals.

In an experimental study, drugs are used to try to block reabsorption in the kidneys and the gut. We want to investigate to what extent these drugs can enhance excretion. We are collaborating with researchers in Denmark, who are conducting a similar study.

Other PFAS studies involving the research group

2020

Nielsen C, Andersson Hall U, Lindh C, Ekström U, Xu Y, Li Y, Holmäng A, Jakobsson K Pregnancy-induced changes in serum concentrations of perfluoroalkyl substances and the influence of kidney function. Environ Health. 2020 Jul 8;19(1):80. doi: 10.1186/s12940-020-00626-6. PMID: 32641055

Measured PFAS levels in the blood change during pregnancy. In this study, we compared PFAS levels in blood samples taken during early and late pregnancy in a group of women with background exposure to PFAS. Such knowledge is needed to be able to compare results in different studies of e.g. birth weight.

Xu Y, Fletcher T, Pineda D, Lindh CH, Nilsson C, Glynn A, Vogs C, Norström K, Lilja K, Jakobsson K, Li Y. Serum Half-Lives for Short- and Long-Chain Perfluoroalkyl Acids after Ceasing Exposure from Drinking Water Contaminated by Firefighting Foam. Environ Health Perspect. 2020 Jul;128(7):77004. doi: 10.1289/EHP6785. Epub 2020 Jul 10.

High exposure to PFAS in drinking water was detected at Arvidsjaur airport in August 2020. We had a unique opportunity to immediately and repeatedly measure blood levels of many PFAS compounds over six months. This allowed us to calculate the half-life of PFAS compounds with short carbon chains that had been present in the drinking water. These compounds are rapidly excreted.

Silva AV, Ringblom J, Lindh C, Scott K, Jakobsson K, Öberg M. A Probabilistic Approach to Evaluate the Risk of Decreased Total Triiodothyronine Hormone Levels following Chronic Exposure to PFOS and PFHxS via Contaminated Drinking Water. Environ Health Perspect. 2020 Jul;128(7):76001. doi: 10.1289/EHP6654. Epub 2020 Jul 8.

This study is about using advanced pharmacological models and animal experimental data to calculate the risk of impact on thyroid hormones after long-term exposure to PFAS via drinking water. The study used data on PFAS levels from Ronneby and results from a study on monkeys. The study is of theoretical interest when comparing different ways of modelling risk. However, such models must always be verified with real-life results.

Martinsson M, Nielsen C, Björk J, Rylander L, Malmqvist E, Lindh C, Rignell- Hydbom A. Intrauterine exposure to perfluorinated compounds and overweight at age 4: A case-control study. PLoS One. 2020 Mar 16;15(3):e0230137. doi: 10.1371/journal.pone.0230137. PMID: 32176721; PMCID: PMC7075550.

This study of over 1000 children compared overweight 4-year-olds from Malmö to a normal weight comparison group. No association was found between the mother's PFAS levels during early pregnancy and the children's weight at age 4.

2021

Norén E, Lindh C, Glynn A, Rylander L, Pineda D, Nielsen C. Temporal trends, 2000-2017. Temporal trends, 2000-2017, of perfluoroalkyl acid (PFAA) concentrations in serum of Swedish adolescents. Environ Int. 2021 Oct;155:106716. doi: 10.1016/j.envint.2021.106716. Epub 2021 Jun 16. PMID: 34144476.

Between 2000 and 2017, a total of 1,200 teenagers from southern Sweden provided blood samples for testing, including levels of various environmental pollutants. The levels of PFOS. PFOA and PFHxS declined steadily throughout the period, while levels of PFNA and PFDA only started to fall after 2009.

2022

Aro R, Eriksson U, Kärrman A, Jakobsson K, Yeung LWY. Extractable organofluorine analysis: A way to screen for elevated per- and polyfluoroalkyl substance contamination in humans? Environ Int. 2022 Jan 15;159:107035. doi: 10.1016/j.envint.2021.107035.

This study used blood samples from our biobank. Using advanced chemical methods for screening fluorinated compounds, it was possible to quantify "undetected" PFASs present at background exposure (samples from Karlshamn) and at high PFAS exposure (samples from Ronneby). Such a screening method could be a good tool for environmental monitoring.

Johanson G, Gyllenhammar I, Ekstrand C, Pyko A, Xu Y, Li Y, Norström K, Lilja K, Lindh C, Benskin JP, Georgelis A, Forsell K, Jakobsson K, Glynn A, Vogs C. Quantitative relationships of perfluoroalkyl acids in drinking water associated with serum concentrations above background in adults living near contamination hotspots in Sweden. Environ Res. 2022 Dec 16:115024. doi:10.1016/j.envres.2022.115024.

Report in Swedish: https://www.diva-portal.org/smash/get/diva2:1651713/FULLTEXT03.pdf

In this study, information on PFAS levels in drinking water was combined with advanced pharmacological models of PFAS uptake and excretion. The aim is to be able to calculate the blood levels that may occur in the population at sites with elevated levels of PFASs in drinking water, and thus be able to make risk assessments more quickly.

2023

Stübner C, Nielsen C, Jakobsson K, Gillberg C, Miniscalco C. Early-Life Exposure to Perfluoroalkyl Substances (PFAS) and Child Language and Communication Development: A Systematic Review. Int J Environ Res Public Health. 2023 Dec 12;20(24):7170. doi: 10.3390/ijerph20247170. PMID: 38131721; PMCID: PMC10742458.

This study is a review of investigations on PFAS exposure and neurodevelopment, with focus on speech and language development as a sensitive endpoint. In total, 15 studies were identified, of which 13 at background exposure levels. No systematic effect of early-life PFAS exposure on language and communication development was found. These inconclusive findings may partly be explained by the use of general test instruments with limited validity as to children's language and communication development. Further studies over a wider exposure range using specific language test instruments are needed.

Other PFAS studies of relevance in Ronneby

Mussabek D, Ahrens L, Persson KM, Berndtsson R. Temporal trends and sediment-water partitioning of per- and polyfluoroalkyl substances (PFAS) in lake sediment, Chemosphere 2019,227:624-9.

Mussabek D, Persson KM, Berndtsson, R, AhrensaL, Nakagawa K, Imura T. Impact of the Sediment Organic vs. Mineral Content on Distribution of the Per- and Polyfluoroalkyl Substances (PFAS) in Lake Sediment. Int. J. Environ. Res. Public Health 2020, 17(16), 5642; https://doi.org/10.3390/ijerph17165642

Mussabek D, Söderman A, Imura T, Persson KM, Nakagawa K, Ahrens L, Berndtsson. PFAS in the drinking water source: Analysis of the contamination levels, origin and emission rates. Water 2023, 15(1), 137; https://doi.org/10.3390/w15010137

Studies of PFAS occurrence in soil and water in the Bredåkra Delta from the Division of Technical Water Resources, LTH, Lund University.

Birgersson L, Jouve J, Jönsson E, Asker N, Andreasson F, Golovko O, Ahrens L, Struve J. Thyroid function and immune status in perch (Perca Fluvialis) from lakes contaminated with PFASs and PCB s. Ecotoxicol Environ Saf 2021 Oct 1:222:112495. doi 10.1016/j.ecoenv.2021.112495. epub 2021 Jul 12

Perch fished in a shallow lake in the Kallinge airport area showed an impact on thyroid hormones and immunological markers.

Funding of research within the Ronneby PFAS Research Programme

Initial funding from the healthcare system in the Southern Health Region.
The open sampling in Ronneby for the determination of PFAS content in blood in 2014-2015 was financed in its entirety within the regular activities of the Occupational and Environmental Medicine Clinic in Lund (AMM Syd). The clinic is part of the specialised healthcare system in Skåne, Blekinge, Kronoberg County and southern Halland and is financed by so-called joint financing.

Research funds, received in competition

Emergency grant from FORMAS, 216-2014-1709
Exposure to PFAS in drinking water - a study in Ronneby municipality
4,89 MSEK, 2014-20216 Project leader. Kristina Jakobsson, Lund University.

File number FORTE 2015-00166
Exposure to perfluorinated substances in drinking water and the health of pregnant women, birth outcomes and growth of children up to 7 years of age.
Project leader: Anna Rignell-Hydbom, Lund University.
2.03 MSEK, 2016-2018

File number FORTE 2015-00732
High exposure to perfluorinated substances in drinking water - a study using well-known and new biomarkers for the effects of perfluorinated substances in drinking water
Project leader: Kristina Jakobsson, Lund University.
2.98 MSEK, 2016-2018

File number ALFGBG-539921
High exposure to perfluorinated substances in drinking water - a study with well-known and new biomarkers of effect
Project leader: Kristina Jakobsson, University of Gothenburg.
0.9 MSEK, 2016-2017

File number FORTE 2016-00155
Modelled exposure to perfluorinated alkyl acids from drinking water in Brantafors, Ronneby (Sweden)
Project leader: Kristina Jakobsson, University of Gothenburg.
3.60 MSEK, 2017-2019

File number VR 2016-01003
Health effects of exposure to perfluorinated substances during pregnancy and early life - the role of epigenetics
Project leader: Karin Engström, Lund University.
2.80 MSEK, 2017-2020

File number ALFGBG-673911
High exposure to perfluorinated substances in drinking water - a study with well-known and new biomarkers of effect
Project leader: Kristina Jakobsson, University of Gothenburg
0.05 MSEK, 2017

File number FORMAS 2017-00875
Half-life of linear and branched perfluorinated alkyl acids after cessation of exposure through drinking water
Project leader: Kristina Jakobsson, University of Gothenburg.
2.63 MSEK, 2018-2020

File number FORMAS 2017-00875
Renal and gastrointestinal excretion of perfluorinated alkyl acids - a study of mechanisms and half-life
Project leader: Tony Fletcher, London School of Hygiene and Tropical Medicine and University of Gothenburg
2.70 MSEK, 2018-2020

File number FORMAS 2017-00896
Transmission of perfluorinated alkyl acids from mother to child during pregnancy and lactation after long-term drinking water contamination in Ronneby, Sweden.
Project leader: Christel Nielsen, Lund University.
2.99 MSEK, 2018-2020

Reference number ALFGBG-774131
High exposure to perfluorinated substances in drinking water - a natural experiment in Ronneby, Sweden
Project leader: Kristina Jakobsson, University of Gothenburg
0.25 MILLION, 2018

File number FORTE 2018-00389
A population-based study on language development in children after exposure to per- and polyfluorinated substances (PFAS).
Project leader: Carmela Miniscalco Mattsson, Region Västra Götaland/University of Gothenburg.
3.03 MSEK, 2019-2021

File number FORTE 2018-00588
Does exposure to perfluorinated substances increase the risk of common chronic diseases related to metabolic syndrome?
Project leader: Ying Li, University of Gothenburg.
1.43 MSEK, 2019-2020

File number FORMAS 2019-02344
Ronneby mother-child cohort: securing a globally unique infrastructure for PFAS research
Project leader: Christel Nielsen, Lund University.
1.47 MSEK, 2019-2020

File number FORTE 2019-00601
Does PFAS affect antibody responses after vaccination according to the Swedish childhood vaccination programme?
Project leader: Kristina Jakobsson, University of Gothenburg.
1.2 MSEK, 2020-2021

File number VR 2019-01343
Elimination of perfluorinated alkyl acids - observational and human experimental studies
Project leader: Kristina Jakobsson, University of Gothenburg.
2.4 MSEK, 2020-2022

Funding from the Sahlgrenska Academy, University of Gothenburg, for a project-bound PhD position starting in 2020 for the project Elimination of perfluorinated alkyl acids after completion of external exposure (60% salary funding for PhD student for 4 years). Supervisor: Kristina Jakobsson, University of Gothenburg.

File number FORTE 2020-00112
Immunotoxicity in persons highly exposed to PFAS in Ronneby - immunosuppression, hypersensitivity and autoimmunity.
Project leader: Anna Saxne Jöud, Lund University.
4.37 MSEK, 2021-2023

File number Crafoordska Foundation 202005096
Ronneby mother-child cohort: Establishment of a unique research database for the studies on health effects in children after exposure to high levels of the environmental pollutant PFAS
Project leader: Christel Nielsen, Lund University.
400 kSEK, 2020

The Royal Physiographic Society
Ronneby mother-child cohort - creation of a unique research database to study health effects in children after exposure to high levels of the environmental pollutant PFAS.
Project leader: Christel Nielsen, Lund University.
85 kSEK, 2020

File number Crafoordska Foundation 20210546
Ronneby mother-child cohort: establishing a unique research database to study health effects in children after early exposure to high levels of the environmental pollutant PFAS.
Project leader: Christel Nielsen, Lund University.
400 kSEK, 2021

Interreg Öresund-Kattegat-Skagerrak, NYPS 20303383
Occupational and Environmental Exposure and COVID-19 Research (EXCOVER) partnership.
Project leader: Sandra Søgard Tøttenborg, Bispebjerg and Frederiksberg Hospital, Capital Region of Denmark, University of Copenhagen. 75% of 552790 EUR, 2021.

File number ALFGBG-965901
Perfluorinated substances (PFAS) - persistent environmental pollutants with immune effects,
Project leader: Kristina Jakobsson, University of Gothenburg
1.2 MSEK, 2022-2024

File number Crafoordska Foundation 20220587
High exposure to the environmental pollutant PFAS and the health of pregnant women
Project leader: Christel Nielsen, Lund University.
400 kSEK, 2022

File number FORMAS 2022-01421
Placenta as a new target organ for PFAS toxicity - epidemiological studies of pregnancies in Ronneby, Sweden
Project leader: Christel Nielsen, Lund University.
3 MSEK, 2023-2025

Reference number European Commission: Marie Skłodowska-Curie Actions Postdoctoral Fellowship 101058697. Developmental toxicity of perfluoroalkyl substances (PFASs) in a population of highly exposed children
Fellow: Annelise Blomberg. Supervisor: Christel Nielsen, Lund University.
2.23 MSEK, 2022-2024